STUDIES ON PARTIALLY HYDROLYZED DEXTRAN WITH SPECIAL REFERENCE TO ITS USE FOR PLASMA VOLUME DETERMINATIONS IN MAN

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Studies on Partially Hydrolyzed Dextran With Special Reference to Its Use for Plasma Volume Determinations in Man

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Introduction

The main purpose of the present investigations was to estimate and compare the plasma volume in man obtained by intravenous infusion of partially hydrolyzed dextran with that by the Evans blue method. It soon became obvious that the dextran preparation to be used ought to possess certain qualities, both from physico-chemical and serological points of view. Therefore, different dextran fractions were studied and the occurrence of dextran-reacting antibodies and related problems were investigated. In addition, a simple method for the determination of dextran was introduced, suitable for clinical routine work.

This dissertation is based on the following papers:

I. JACOBSSON, L., and ZSIGA, J., Dextran-reacting antibodies in human serum. *Acta Soc. Med. Upsal. 63,* 165 (1958).

II. JACOBSSON, L., and WIKSTRÖM, R., The detection of dextran-reacting antibodies in human serum. *Acta Soc. Med. Upsal. 63,* 180 (1958).

III. JACOBSSON, L., and ZSIGA, J., The effect of intravenous infusion of dextran on blood pressure in Leuconostoc-immunized rabbits. *Acta Soc. Med. Upsal. 63,* 188 (1958).

IV. JACOBSSON, L., Intravenous infusion of clinical B 512 dextran in normal human subjects with dextran-reacting antibodies in serum. *Acta Soc. Med. Upsal. 63,* 196 (1958).

V. JACOBSSON, L., and WIKSTRÖM, R., Disappearance rate of ^{51}Cr-labeled clinical dextran from blood in man. *Acta Soc. Med. Upsal. 63,* 204 (1958).

VI. JACOBSSON, L., and HANSEN, H., A simple method for determination of dextran in blood and urine. *Scand. J. Lab. Clin. Invest. 4,* 352 (1952).

VII. JACOBSSON, L., Determination of low dextran concentrations in human plasma and urine. *Acta Soc. Med. Upsal. 63,* 212 (1958).

VIII. JACOBSSON, L., The determination of plasma volume in man. A comparison of the dextran and the Evans blue dye spaces. *Acta Soc. Med. Upsal. 64,* 1 (1959).

The Roman figures above are used as references to the respective papers.

General Remarks

It was shown by Grönwall and Ingelman (1944, 1945) that partial hydrolysis of native dextran removed its toxic properties. By fractionation procedures preparations were obtained which made dextran suitable as a plasma expander (clinical dextran). The molecular weight distribution was kept within restricted ranges whereby the dextran remained in blood for an appropriate time. This fact animated Birch *et al.* (1953) to use dextran for the determination of blood volume in man. However, untoward reactions to the injection of dextran appeared in 20 per cent of the cases and the method for the determination of dextran made the procedure too laborious for routine use. This latter fact was reduced by the use of a simple turbidimetric method (Paper VI). In a small series of preliminary experiments (Jacobsson and Hansen, 1954) this method was adapted in a modified form for plasma volume determinations using a Swedish dextran preparation. No undesired reactions were observed but a considerable amount of dextran was eliminated via the kidneys and the procedure for the determination of dextran was less satisfactory. Therefore the turbidimetric method for the determination of dextran has been improved (Paper VII) and more sharply fractionated dextran preparations have been investigated (Paper VIII). The plasma volume obtained has been compared with the one simultaneously estimated by the Evans blue technic. Recently some papers concerning plasma volume determinations in man, where various high molecular weight dextrans were used, have been published by Bloom (1956), Jaenike *et al.* (1956, 1957) Craig and Waterhouse (1957), and Semple and Thomsen (1957).

The suggestions by Hehre and Sugg (1950) that undesired reactions on the infusion of clinical dextran in man might originate from the antibody-combining properties of dextran, initiated further investigations on this and resemblant questions. The frequency of naturally occurring dextran-reacting antibodies in human serum was studied in normal subjects and in patients (Paper I), according to the qualitative

technic by Swift *et al.* (1943). This method was compared with the quantitative one by Heidelberger and MacPherson (1943) in Paper II. The effect of intravenous infusion of dextran on subjects with dextran-reacting antibodies in serum was investigated, in the first place, on rabbits immunized with *Leuconostoc mesenteroides* vaccine (Paper III) and later on normal human subjects with naturally occurring dextran-reacting antibodies in serum (Paper IV) with clinical dextran. The eventual influence of the presence of dextran-reacting antibodies in human serum on the plasma dextran level has been investigated by the use of ^{51}Cr-labeled dextran (Paper V) and attention has been paid to this possibility in Paper VIII.

The Influence of the Molecular Weight Distribution of the Dextran Preparation

Clinical dextrans have controlled but rather wide molecular weight distributions. For modern Swedish types the weight average molecular weight as measured with light scattering method is 80,000 ± 10,000; for 10 per cent low molecular weight fraction not less than 25,000 and not more than 200,000 for the 10 per cent high fraction (Grönwall, 1957). Preliminary investigations on the determination of plasma- and blood volume with clinical dextrans and preparations that contained a substantial part of low molecular weight material showed that too high values were obtained when compared with current methods, partly due to a loss of the test substance via the kidneys (Birch *et al.*, 1953; Jacobsson and Hansen, 1954; Craig and Waterhouse, 1957). These results are in accordance with the investigations on the glomerular permeability for dextran by several authors, among others Ingelman and Halling (1949), Brewer (1951), Grönwall *et al.* (1952), Wallenius (1954), and Giebisch *et al.* (1954). They showed that the renal elimination of dextran decreased with increasing molecular weight and that the recovery of dextran from urine could roughly be calculated from the molecular weight distribution curve of the preparation used. In normal humans the mean maximal molecular weight of dextan in urine was about 46,000 after the administration of dextran intravenously (Wallenius, 1954), but in patients with increased capillary permeability figures somewhat exceeding 100,000 were found. By using dextran preparations where even the low weight fraction had a molecular weight well above that of albumin the renal elimination can be reduced to negligible amounts. Craig and Waterhouse used a preparation with an average molecular weight of 194,900 and with 4.8 per cent low fraction of 129,000. They found in normal subjects that only minute amounts were recovered from urine −0.7 and 1.5 per cent of the injected dose, during 1 and 4 hours respectively after the injection. In a

case with albuminuria the renal elimination was not elevated. Similar results were obtained by Jacobsson (Paper VIII) with two different dextran preparations. Although the average molecular weight of the first one, Dextran II (222,000), was considerably higher than the second, Dextran III (143,000), the urinary excretion of the former was somewhat higher—in patients on an average 0.59 and 0.10 per cent of the given dose during the first 15–20 minutes after the injection. The highest amount excreted was 5.9 of Dextran II and 0.42 per cent of Dextran III. This fact might be explained by a broader molecular weight distribution and a larger part of low molecular weight material of the first preparation. It is thus of primary importance that the dextran preparation used should contain a negligible amount of material that can rapidly leave the blood plasma, even in cases with increased capillary permeability.

However, fractions with very high molecular weights have to be excluded as well. As primarily shown by Grönwall and Ingelman (1945) native dextran when administered intravenously will cause thrombosis of the renal and the liver capillaries. Partially hydrolyzed dextrans of high molecular weights might also provoke undesired reactions (Thorsén, 1954; Kabat *et al.*, 1957).

Therefore, a suitable dextran preparation for plasma determinations ought to have a moderately high average molecular weight and a narrow molecular weight distribution.

The Degree of Branching of the Dextran

Chemical analyses of dextrans from a large number of bacterial strains by Jeanes *et al.* (1954) showed that the content of α-glucosidic linkages of different types (1:6, 1:4, 1:3) varied considerably between the different strains. An increasing content of non 1:6 linkages was supposed to be evidence of an increasing content of branching. Clinical investigations with partly hydrolyzed dextran of varied degree of branching have shown that untoward reactions appeared less frequently on the infusion of dextrans with a low content of non 1:6 linkages (Pulaski 1951; Amspacher and Artz, 1951; Tarrow and Pulaski, 1953; Wilkinson, 1956; Kabat *et al.*, 1957). These authors found the dextran produced by the B 512 strain of *Leuconostoc mesenteroides* to be the substance of choice. Analyses of clinical samples of this dextran from three different factories by Moyer and Isbell (1957) showed a high content of 1:6 linkages (92.3–93.8 per cent). The B 512 dextran used in Papers I–VIII contained 94 per cent 1:6 linkages (Granath, 1958). Although rather high molecular weight preparations were used for the determination of plasma volume the frequency of untoward reactions to the injection of dextran was less than 2 per cent (Paper VIII). Birch *et al.* (1953), by using an older type of clinical dextran for the

estimation of blood volume in man, reported undesired effects in 5 out of 25 cases. Dextran with a low content of branching seems therefore to be preferred.

The Nature and Origin of the Dextran-Reacting Antibodies

In rabbits, partially hydrolyzed dextrans adsorbed on haemolytic streptococci gave rise to two different types of antibodies to dextran (Glynn, Holborrow and Johnson, 1954). With the same technic as used in Paper I the optimal dextran concentration for the one was 10^1–10^2 μg per ml and for the other 10^2–10^4 μg per ml. Immunization of rabbits by intravenous injections of Leuconostoc mesenteroides B 512 vaccine gave rise to antibodies which reacted optimally at a dextran concentration of 10^2 μg per ml (Paper I). For naturally occurring antibodies in human sera, however, the corresponding figure was 10^4–10^5 μg dextran per ml. This fact indicates that the rabbit antibodies and those in human sera were of separate types.

The occurrence of at least two separate types of dextran-reacting antibodies in man has been revealed by Kabat and Berg (1953). Oligosaccharides with 1:6 linkages inhibited the one type of antibodies and 1:4 oligosaccharides the other. Evidently there were antibodies also specific to 1:3 linkages (Kabat, 1954). The optimal dextran concentration in the quantitative precipitin test according to Heidelberger and MacPherson (1943) was definitely lower for the antibodies of the 1:6 type than for those of non 1:6 type. Therefore, the antibodies detected by the qualitative method were supposed to be directed mostly against non 1:6 linkages.

It has been shown by Kabat *et al.* (1957) that repeated intravenous infusions of clinical dextran did not increase the amount of nitrogen precipitable by dextran. Thus the use of clinical dextran as a plasma expander is not the cause of the occurrence of dextran-reacting antibodies in serum. Kabat and Berg (1953) suggested an immunization via the alimentary tract. This statement was based on the fact that commercial sugar might contain dextran (Neill *et al.*, 1939) and that dextran producing bacteria in the alimentary tract were isolated by Hehre and Neill (1946). The crossreactivity of dextrans with various antibodies offers ample possibilities to explain the findings of dextran-reacting antibodies in human serum; this is reviewed in Paper I.

The Occurrence of Dextran-Reacting Antibodies in Human Serum

Naturally occurring antibodies in human serum combining with different dextrans have been revealed by severad authors (Kabat and

Berg, 1952, 1953; Maurer, 1953; Grabar, 1955; Jacobsson and Zsiga, Paper I; Jacobsson and Wikström, Paper II), and with *Leuconostoc mesenteroides* by Warren *et al.* (1952) and Jacobsson and Zsiga (Paper I). By using the capillary tube technic by Swift *et al.* (1943) sera from 845 healthy young men and from 852 hospitalized persons of both sexes with various disorders were investigated. Positive reactions to clinical B 512 dextran were found in 4 to 5 per cent and to Leuconostoc mesenteroides of the strain B 512 in about 7 per cent. No differences in frequency were obtained between the normal and the hospitalized subjects (Paper I). Warren *et al.* found that 9 sera of 53 normal persons agglutinated *L. mesenteroides*. The strain used however was not communicated. Leuconostoc vaccine that contains native dextran was thus more sensitive than clinical dextran in detecting antibodies. Kabat (1954) has shown that the size of an antibody combining area of the dextran molecule was at least that of a trisaccharide. The number of such uninterrupted units of non 1:6 linkages might be reduced by splitting during the manufacturing process. In fact, experiments with antibodies in human serum and dextran fractions of various molecular weights showed that the precipitation tests were strongest for the highest molecular weight fraction and then decreased gradually as the molecular weights diminished (Paper I). A comparison between the qualitative method used in Paper I with the quantitative one by Heidelberger and MacPherson (1943) showed a good correlation (Paper II).

The Effect of Intravenous Infusion of Dextran on Subjects with Dextran-Reacting Antibodies in Serum

Hehre and Sugg (1950) have suggested that undesired reactions on the infusion of clinical dextran in man might originate from the antibody-combining properties of dextran. However, in a preliminary study on rabbits immunized by intravenous injections of Leuconostoc mesenteroides vaccine no blood pressure fall or any sign of anaphylactic reaction was observed when clinical dextran was given intravenously (Paper III). In man, Kabat *et al.* (1957) could demonstrate significant correlations between the amount of nitrogen in serum precipitable by dextran and systemic reactions to certain clinical dextrans in normal individuals. Thus 4 out of 6 subjects with a high antibody level showed untoward effects on the infusion of a B 512 clinical dextran preparation. In a series of 44 young men, whose sera contained Leuconostoc-reacting antibodies and in 35 cases also antibodies to dextran, the intravenous infusion of 500 ml 6 per cent B 512 dextran of modern Swedish clinical type provoked undesired reactions in 25 per cent of the whole material and in 31 per cent of the subjects with dextran-reacting

antibodies (Paper IV). These side-effects were mostly mild and might easily have been overlooked. In two cases more pronounced symptoms appeared; both of these subjects had a history of allergic disorders. When using high molecular weight preparations of a B 512 dextran for the determination of plasma volume (Paper VIII) not one of the 22 subjects whose sera contained dextran-reacting antibodies showed any sign of undesired side-effects. The presence of dextran-reacting antibodies in serum seems not to be the explanation of the occurrence of undesired reactions on the infusion of dextran, but in some cases with allergic disorders.

The Determination of Dextran when Used for the Evaluation of Plasma Volume

As a rule, rather small amounts of dextran are infused when used for plasma volume determinations. Therefore, a micromethod is required for the estimation of the dextran concentration in plasma. Most authors have used some modification of the anthrone method, Birch *et al.* that of Bloom and Willcox (1951) which includes treatment with alkali and precipitation of the dextran by alcohol; Bloom used the same method with the modification of using trichloracetic acid for deproteinization; Craig and Waterhouse, and Jaenike *et al.* used another modification of this method including recentrifugation of the redissolved precipitated material, whereby the blank value was considerably reduced. Semple and Thomsen used a modification (Semple, 1957) which included deproteinization with trichloracetic acid and dialysis of the supernatant for glucose removal. All these modifications of the anthrone method might increase the specificity of the procedure, but also make it too time-consuming and laborious for routine use.

The turbidimetric method according to Jacobsson and Hansen (Paper VI) was originally designed for the determination of dextran when used as a plasma volume expander. This procedure has been modified (Paper VII) to cover the range of dextran concentrations in plasma which will be found when about 1000–2000 mg dextran has been given for the estimation of the plasma volume, i.e. 30–120 mg per 100 ml. The method involves the precipitation of dextran by alcohol under controlled conditions after deproteinization by trichloracetic acid. The turbidity obtained is read in a photometer. The procedure is completed within one hour. The method is not specific to dextran, as any substance will interfere which is non-precipitable by trichloracetic acid and precipitable by alcohol under the given conditions. This effect is reduced by subtracting the blank, determined on a specimen taken before the infusion of dextran, from the final turbidity. The standard error of

a single determination was 0.93 mg per cent when calculated from double determinations of 50 plasma samples with a dextran concentration varying from 50 to 100 mg per cent.

On the Technic Used for the Determination of Plasma Volume and Results in Normal Subjects

About 900 mg and 2000 mg of Dextran II and of Dextran III respectively and about 10 mg of Evans blue dye were injected by using special, calibrated syringes of the same type as described by Wiklander (1956) and an Olovson needle for the vein puncture. Saline was injected simultaneously by an assistant to flush all the test substance into the vein. Samples were taken before the injection and 15 minutes later according to von Porat (1951) and Wiklander (1956). Oxalated plasma was used for dextran determinations and serum for Evans blue dye.

The accuracy of the dextran method was estimated from double determinations on 20 healthy subjects at intervals of six to eight weeks. The standard error of a single determination was 103 ml. corresponding to 4 per cent of the mean plasma volume.

The mean plasma volume of 10 healthy young men when using Dextran II was 3344 ml and with Evans blue 3234 ml. In 20 normal males the Dextran III mean plasma volume was 2943 ml with a standard error of the mean of ± 69 ml and a standard deviation of 308 ml. The corresponding figures for Evans blue was 3362 ± 85 and the standard deviation 381 ml. In 40 normal females the mean plasma volume with Dextran III was 2385 ± 43 ml and a standard deviation of 271 ml and with Evans blue 2640 ± 51 ml and 321 ml respectively.

The coefficients of correlation between weight, length, body surface area and the Dextran III plasma volume were $+0.78 \pm 0.05$; $+0.73 \pm 0.06$ and $+0.80 \pm 0.05$ respectively. The difference between the coefficients was not significant.

Comparison of the Dextran and the Evans Blue Method for the Determination of Plasma Volume in Man

The investigations by Birch *et al.* on blood volume when using dextrans of clinical types showed that the difference between dextran and Evans blue mean blood volumes was +11 per cent (expressed in per cent of the dextran mean blood volume). Jacobsson and Hansen found a difference of +21 per cent between the plasma volumes of the two methods. They used a dextran preparation of a weight average molecular weight of 312,000 and 11 per cent of less than 45,000. Three different dextran preparations were studied by Bloom. The first one

had an average molecular weight of 194,900, the second 255,000 and the third 412,000. The first contained 4.8 per cent low fraction of 129,000; further specifications were not given for the other ones. The difference between the dextran and the Evans blue method, calculated as above, was -7, -10 and -4 per cent, respectively. The first two differences were of borderline significance ($P=0.05$), but the third one was not significant ($0.4>P>0.3$). In this case, however, the series was small and comprised only 7 subjects. Jaenike *et al.* used the ^{131}I-albumin method as a reference. As the iodine-tagged albumin and the Evans blue methods have been shown to give comparable values for plasma volume (Schultz *et al.*,1953; Freinkel *et al.*, 1953; Inkley *et al.*, 1955), it seems to be justified to include the results by Jaenike *et al.* They used a dextran preparation of an average molecular weight of 194,900 and 4.8 per cent low fraction of 129,000—evidently the same as the first one used by Bloom. In normal subjects the difference averaged -6 per cent and about -10 per cent in patients with congestive heart failure and edema. The mean difference between the two methods was statistically significant ($P<0.01$). Semple and Thomsen used a high molecular weight fraction of clinical dextran. No data were given on this preparation, but it was not excreted in the urine in appreciable amounts and left the circulation only very slowly. The mean difference between the dextran and the Evans blue methods was $+3$ per cent, however not significant. In Paper VIII two high molecular weight preparations of Swedish B 512 dextran have been investigated. The fihst one, Dextran II, had an average molecular weight of 222,000 and 12 per cent low fraction of less than 93,000. The corresponding figures for the second preparation, Dextran III, was 143,000 and for 7.2 per cent low fraction 93,000. With Dextran II the mean difference between the dextran and the Evans blue method amounted to $+3$ per cent in normal young men and $+5$ per cent in hospitalized subjects with various disorders. The mean difference between the two methods for the normal cases was not statistically significant ($P>0.05$) but for the patients significant ($0.001<P<0.01$). If all the cases were taken together a significant difference was obtained ($P=0.01$). With Dextran III the difference for normal males was — 12 per cent and for normal females -11 per cent. These differences are highly significant ($P<0.001$). For the patients the difference was -6 per cent, but less significant ($0.01<P<0.02$) than for the normal group. When all the cases were taken together, however, the difference between the mean values was highly significant ($P<0.001$). The mean differences when not communicated have been calculated from the figures given by the authors. The results of the investigations mentioned are summarized in Table 1. As is seen, the mean difference between the dextran and the reference method varies from $+21$ to -12 per cent. These figures are not correlated to the average molecular weight of the preparation

Table 1. *Simultaneous determination of PV[a] with dextran and with Evans blue ([131]I-albumin respectively) made by various authors.*

The per cent difference was calculated as the difference between Evans blue PV ([131]I-albumin resp.) and dextran PV, divided by the dextran PV and multiplied by 100.

Author	Material	Dextran preparation				
		No. of cases	Av. molec. weight	Amount injected (mg)	Ref. method	% diff. of mean values
Birch *et al.*, 1953	P[b]	20	Clin. types	3600–6000	Ev. blue	+11
Jacobsson & Hansen, 1954	P	10	312,000	1200	Ev. blue	+21
Bloom, 1956	P	16	194,900	3000?	Ev. blue	− 7
	P	12	255,000	3000?	Ev. blue	−10
	P	7	412,000	3000?	Ev. blue	− 4
Jaenike *et al.*, 1956	♂ N[b]	10	194,900		[131]I-alb.	− 6
	P	13	194,900		[131]I-alb.	−10
Jaenike *et al.*, 1957	♂ N	10	194,900	2130–2400	[131]I-alb.	− 6
	P	15	194,900	2130–2400	[131]I-alb.	−10
	P	8	194,900	2130–2400	[131]I-alb.	− 9
Semple & Thomsen, 1957	N		High-molec. type		Ev. blue	+ 3
Jacobsson, 1959	♂ N	10	222,000	900	Ev. blue	+ 3
	P	57	222,000	900	Ev. blue	+ 5
	♂ N	20	143,000	2000	Ev. blue	−12
	♀ N	40	143,000	2000	Ev. blue	−11
	P	69	143,000	2000	Ev. blue	− 6

[a] PV = plasma volume.
[b] P = patients; N = normals.

used as the amount of low molecular weight fraction that can rapidly leave the blood plasma will be of primary importance for the value of the plasma volume.

Advantages and Drawbacks of Dextran in Comparison with Evans Blue for the Determination of Plasma Volume

There are some difficulties in the estimation of plasma volume with Evans blue which are avoided when using the dextran method. Hemolysis and lipemia might introduce considerable errors into the Evans blue method. This might be reduced by several procedures (for references see Wiklander, 1956) which, however, are less suitable in clinical routine. Of less importance might be the variation in the absorbancy of the same amount of dye in the plasma of different subjects (Wiklander, 1956). The occurrence of varying amounts of impurities of the different dye preparations will also influence the results as the

disappearance rate of the impurities from plasma has been shown to be very rapid (Cooley, 1954).

In some disease-states the use of high molecular weight dextran is to be preferred to Evans blue. In patients with highly icteric sera the Evans blue dye injected is less completely bound to the albumin and the rapid loss of free dye causes an erroneously high plasma volume figure. Conditions in which increased capillary permeability is present will cause an overestimation of the plasma volume as determined with Evans blue or iodine-tagged albumin (Jaenike *et al.*, 1957). This error can be reduced by using a high molecular weight dextran preparation where even the lowest molecular weights can be kept at a level well above that of albumin.

The widespread use of Evans blue for the determination of plasma volume is partly due to the simple determination of the dye. Although dextran is superior to Evans blue in many respects, most methods for the determination of dextran are rather laborious and less suitable in clinical routine. However, the turbidimetric method of Paper VII offers a procedure simple enough for routine work.

On the intravenous injection of dextran untoward reactions appeared in a few cases. They were of a mild to a moderate degree and probably did not influence the plasma volume determination. The occurrence of systemic reactions to Evans blue dye in man have been emphasized by Roberts (1954) who considered the substance to be a dangerous and unsatisfactory agent for the performance of serial blood volume determinations in human subjects. These statements do not agree with my experience of this substance. Hueper and Ichniowski (1944) have shown that in animals the dye was retained in various organs and tissues, but dextran was only transitorily deposited and disappeared without leaving any traces (Persson, 1952).

For normal males the coefficient of variation was 10 and for normal females 11 for plasma volume determinations with dextran. The corresponding figures with Evans blue were 11 and 12 respectively. From this point of view there is equality between the two methods.

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